1    **High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of**

2    **deaths in urban Malawi**

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21    **Key Words:** SARS-CoV-2, COVID-19, Malawi, Seroprevalence, IgG

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24

25  **Abstract**

26  **Background**: In low-income countries, like Malawi, important public health measures

27  including social distancing or a lockdown have been challenging to implement owing to

28  socioeconomic constraints, leading to predictions that the COVID-19 pandemic would

29  progress rapidly. However, due to limited capacity to test for severe acute respiratory

30  syndrome coronavirus 2 (SARS-CoV-2) infection, there are no reliable estimates of the true

31  burden of infection and death.  We, therefore, conducted a SARS-CoV-2 serosurvey amongst

32  health care workers (HCWs) in Blantyre city to estimate the cumulative incidence of SARS-

33  CoV-2 infection in urban Malawi.

34  **Methods**: We recruited 500 otherwise asymptomatic HCWs from Blantyre City (Malawi)

35  from 22nd May 2020 to 19th June 2020 and serum samples were collected from all

36  participants. A commercial ELISA was used to measure SARS-CoV-2 IgG antibodies in

37  serum.

38  **Results**: A total of 84 participants tested positive for SARS-CoV-2 antibodies. The HCWs

39  with positive SARS-CoV-2 antibody results came from different parts of the city. The

40  adjusted seroprevalence of SARS-CoV-2 antibodies was 12.3% [CI 8.2 - 16.5]. Using age-

41  stratified infection fatality estimates reported from elsewhere, we found that at the observed

42  adjusted seroprevalence, the number of predicted deaths was eight times the number of

43  reported deaths.

44  **Conclusions**: The high seroprevalence of SARS-CoV-2 antibodies among HCWs and the

45  discrepancy in the predicted versus reported deaths suggests that there was early exposure but

46  slow progression of COVID-19 epidemic in urban Malawi. This highlights the urgent need

47  for development of locally parameterised mathematical models to more accurately predict the

48  trajectory of the epidemic in sub-Saharan Africa for better evidence-based policy decisions

49  and public health response planning.

**Introduction**

Coronavirus disease 2019 (COVID-19) has had a dramatic impact worldwide, with high mortality in Asia, Europe and the Americas (1). Africa reported its first COVID-19 case on 14th February 2020 (2). Due to poor socio-economic conditions, high HIV prevalence, an increase in non-communicable diseases and challenged health system infrastructure, it was predicted that the African pandemic would progress rapidly. As of 16th July 2020, however, the number of COVID-19 cases was 665,522 and deaths 14,434 (1, 2), much lower than predicted by mathematical models (3).

In low-income countries, like Malawi, important public health measures like social distancing or a lockdown are difficult to implement owing to socioeconomic constraints. Furthermore, the limited capacity to test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection impedes effective public health response planning. Initial testing in Malawi focused on case identification in patients with COVID-19-like symptoms, contacts of index patients and inbound travellers. The first COVID-19 case in Malawi was reported on 2nd April and as of 16th July 2020 there were 2716 cases with only 51 deaths reported (2, 4). Given the sampling strategy, the true burden is certainly much greater than the reported cases, but there are no reliable estimates of the true burden of infection and death. Up to now, health services have reported only small number of cases and have not been overwhelmed as predicted (3).

The unrestricted nature of the COVID-19 epidemic in Malawi provides an opportunity to compare its trajectory in a low-income setting with what has been reported in high income settings. It has been shown that the rate of asymptomatic SARS-CoV-2 infection among health care workers (HCWs) reflects general community transmission rather than in-hospital exposure (5). We, therefore, conducted a SARS-CoV-2 serosurvey amongst HCWs in

3

75    Blantyre city to estimate the cumulative incidence of SARS-CoV-2 infection in urban

76    Malawi.

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78    **Methods**

79    *Ethical statement*

80    Ethical approval was provided by the College of Medicine Research and Ethics Committee

81    (COMREC, Malawi) (P.05/20/3045) and Liverpool School of Tropical Medicine (LSTM,

82    UK) (20-043). All participants gave informed written consent.

83

84    *Study setting and participants*

85    Participant recruitment was done at the Malawi-Liverpool-Wellcome Trust Clinical Research

86    programme (MLW) in Blantyre, Malawi, as part of an ongoing longitudinal study that seeks

87    to investigate markers of SARS-CoV-2 exposure and immunological protection in Malawian

88    adults. The study site is within the compound of the Queen Elizabeth Central Hospital

89    (QECH), the largest tertiary teaching hospital in Malawi. It is easily accessible from most

90    parts of the city. Participants were HCWs from Blantyre City, both clinical and non-clinical.

91    We used a convenience sampling approach, whereby the study was advertised electronically

92    and by word of mouth. The sample size was calculated based on the study primary objective,

93    which was to compare the SARS CoV-2 neutralising antibody titers in recovered COVID-19

94    patients compared to SARS CoV-2 antibody positive (asymptomatic/mild) individuals.

95    Inclusion criteria for the study included being an HCW resident in Blantyre, aged between 18

96    and 65 years old, and otherwise symptomatic. The exclusion criterion was withholding

97    consent. Electronic case report forms (eCRFs) were used to collect demographic data

98    including age, gender, place of residence, common mode of transportation, occupation and

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99     involvement in COVID-19 work. The samples used for this manuscript are from the baseline

100    recruitment arm of the asymptomatic group in the main study.

101

102    *Sample collection, processing and experimental setup*

103    Peripheral blood (10ml), 7ml in Sodium Heparin tubes and 3ml in serum separation tubes

104    (SST) (All BD Biosciences), was collected from all study participants using venesection by

105    the study clinical team at the study site (MLW) between 22nd May 2020 to 19th June 2020.

106    Serum was collected from the SSTs by centrifugation at 500g for 8 mins and stored at -80°C.

107    To measure SARS-CoV-2 antibodies, we used a commercial enzyme linked-immunosorbent

108    assay (ELISA) targeting Spike (S2) and Nucleoprotein (N) from SARS-CoV-2 (Omega

109    diagnostics, UK; ODL 150/10; Lot #103183). The assay was performed as per the

110    manufacturer's instructions. In brief, participant serum was diluted (1:200) in sample diluent

111    (150mM Tris-buffered saline, pH 7.2 with antimicrobial agent). The diluted samples, diluent

112    alone (negative control), manufacturer's cut-off control and positive control were added at

113    100μl per well. The plate was incubated at room temperature for 30 mins. After incubation,

114    the plate was washed three times with wash buffer (100mM Tris-buffered saline with

115    detergent, pH 7.2) using a plate washer (Asys Atlantis, Biochrom Ltd, UK). Human IgG

116    conjugated to horseradish peroxidase was then added to each well at 100μl and incubated for

117    30 minutes at room temperature.  After incubation, the plate was washed four times with

118    wash buffer, and 100μl of TMB (3,3',5,5'-Tetramethylbenzidine) Substrate (aqueous solution

119    of TMB and hydrogen peroxide) was added. The plate was incubated for 10 minutes at room

120    temperature, before addition of 100μl of Stop Solution (0.25M sulphuric acid). The optical

121    density (OD) of each well was read at 450nm in a microplate reader (BioTek ELx808, UK)

122    within 10 minutes. The assay interpretation was as follows; positive result (OD 0.6),

123    indeterminate result (OD 0.55 to < 0.6) and negative (OD < 0.55)  This assay has undergone

124    rigorous independent validation at the Liverpool School of Tropical Medicine (UK) and St

125    George's University of London (UK) (6).

126

127    *Statistical analysis*

128    Graphical presentation was performed using GraphPad Prism 8 (GraphPad Software, USA).

129    To integrate uncertainty arising from test sensitivity and specificity, we used a method

130    published by Reiczigel *et al,* 2010 (7). The geospatial data was plotted in R (v4.0.0) using

131    ggmap (v3.0.0) (8) and ggplot2 (v3.3.1) (9).

132

133    **Results**

134    *Demographics of study participants*

135    We recruited 500 asymptomatic HCWs with a median age of 31 (range 20-64 years). The

136    average household size for the participants was 4 [confidence interval (CI) 3–5]. Of the 500,

137    331 were clinical HCWs and 169 non-clinical HCWs (Table 1). The clinical HCWs included

138    nurses, medical doctors and clinical officers, while the non-clinical HCWs included

139    clerical/administration, field workers and laboratory scientists. The primary workstation for

140    the HCWs included primary healthcare facilities (35/500), secondary healthcare facilities

141    (291/500), and clinical research facilities (174/500). The majority of the participants were

142    nurses (57%), 41% of all participants were involved in clinical work related to COVID-19

143    and 73% of the total participants used public transport or walking as their main means of

144    transport. The main characteristics of the participants are summarised in Table 1.

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146    *Seroprevalence of SARS CoV-2 antibodies and geospatial location of the antibody positive*

147    *individuals*

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148    84 participants tested positive for SARS-CoV-2 antibodies (Figure 1). After adjusting for test

149    sensitivity and specificity (6, 7), the overall seroprevalence of SARS-CoV-2 antibodies was

150    12.3% [CI 8.2 - 16.5]. This suggests that local transmission was high and that SARS-CoV-2

151    may have been circulating for some time in Blantyre City.

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153    To estimate the potential geographical spread of SARS-CoV-2, we plotted the geographical

154    coordinates of place of residence for the individuals with a positive antibody result on the

155    map of Blantyre City. We found that the HCWs with a positive SARS-CoV-2 antibody result

156    came from different parts of the city (Figure 2). This suggests that SARS-CoV-2 local

157    transmission was likely widespread across the city.

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159    *Crude projections of mortality based on seroprevalence estimates*

160    Using estimates of infection fatality rates from Verity *et al.* (10) and the Malawi population

161    census (11), we estimated the number of deaths that could have occurred at the observed

162    seroprevalence of SARS-CoV-2 antibodies (Table 2). We adjusted the population estimates

163    by inflating them to take into account population annual population growth rate of 2% from

164    2018 to 2020 (11). We assumed that there was a uniform risk of infection at all age groups

165    and that the seroprevalence was similar to the general population.

166

167    The crude estimates suggest that there should have been at least 138 deaths by 19th June

168    2020. However, four weeks following the serosurvey, only 17 COVID-confirmed deaths in

169    Blantyre have been reported by the Public Health Institute of Malawi (4), which is

170    approximately eight times below the predicted deaths. When the seroprevalence is

171    extrapolated to the entire Malawi, it predicts approximately 5,295 COVID-19 deaths, but

172    only 51 deaths have been reported as of 16th July 2020. These crude estimates highlight a

173    discrepancy between the predicted deaths using infection fatality rates from elsewhere and

174    the actual number of reported COVID-19 deaths in Malawi.

175

176    **Conclusions**

177    To our knowledge, this seroprevalence study is the first to report estimates of SARS-CoV-2

178    exposure among HCWs in an African urban low-income setting. It provides insights into the

179    potentially unique trajectory of the COVID-19 epidemic in sub-Saharan Africa (SSA), using

180    data from urban Malawi. We observe a high seroprevalence of SARS-CoV-2 antibodies

181    amongst HCWs. It has been reported elsewhere that HCWs accounted for a high proportion

182    of cases early in the SARS-CoV-2 outbreak when transmission was increasing sharply and

183    personal protective equipment (PPE) provision was patchy (12-14).  Our data could suggest

184    that Malawi is relatively early in the epidemic and that COVID-19 cases are likely to

185    continue to rise sharply in the coming weeks, but the serology also suggests that large

186    numbers of cases must be either asymptomatic or only show mild disease.

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188    The discrepancy between the predicted compared to reported mortality at the observed

189    seroprevalence estimate may also suggest that there are large numbers of underreported or

190    misclassified deaths in Malawi. However, even in countries like South Africa with relatively

191    abundant testing capacity and strong health systems, there is relatively low mortality with a

192    case fatality ratio of 1.5 (15). This may imply that the impact of SARS-CoV-2 in Africa is

193    potentially much less severe or is following a different trajectory than that experienced in

194    China, Americas and Europe, where case fatality ratios were commonly above five (1). This

195    warrants further investigation.

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197     However, the reasons behind the discrepancy in the COVID-19 pandemic trajectory between

198     SSA and elsewhere might include population demography, climate and prior cross-reactive

199     immunity (16). In Malawi, for example, the population is younger (median age 17 years old)

200     (11), and the elderly who mostly experience worse outcomes in other settings (10), are 5.1%

201     of the population (11), largely residing in rural areas. If the prevalence of SARS-CoV-2 is

202     very low in rural areas, this may explain the low number of deaths, and would strengthen the

203     call to shield the elderly (17).

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205     This study has some limitations. First, selection bias is likely due to the convenience

206     sampling approach; however, targeting HCW for regular serosurveys could help predict local

207     transmission outbreaks. Second, this serosurvey focused on an urban population where

208     Malawi has reported the highest concentration of COVID-19 cases. The seroprevalence in the

209     rural population remains unknown, but if high, may prompt other explanations for the

210     African/Malawi situation. Third, current SARS-CoV-2 ELISAs are still undergoing rigorous

211     validation and verification in the African settings, hence seroprevalence estimates could

212     change with new information on the accuracy of the test kits.

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214     In conclusion, our findings indicate a major discrepancy between predicted COVID-19

215     mortality at the observed SARS-CoV-2 seroprevalence in HCWs with reported COVID-19

216     deaths in urban Malawi. The high seroprevalence estimate implies earlier exposure of SARS-

217     CoV-2 than that reported but with slow progression of the COVID-19 epidemic.

218     Development of locally parameterised mathematical models should be prioritised to more

219     accurately predict the trajectory of the epidemic in SSA. This will allow better evidence-

220     based policy decision-making and public health response planning.

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**Data availability**

Figshare: High SARS-CoV-2 seroprevalence in Health Care Workers but relatively low

numbers of deaths in urban Malawi. https://doi.org/10.6084/m9.figshare.12745214.v2

This project contains the following underlying data:

- PROTECT_FigShare Part 1 31072020.csv

- PROTECT_screening_enrolment_data_dictionary Figshare 31072020.pdf

Data are available under the terms of the Creative Commons Attribution 4.0 International

license (CC-BY 4.0).

247

248 **Disclaimer**

249 The funders had no role in the study design, data collection and interpretation, or the decision

250 to submit the work for publication. Mologic (UK) was provided the opportunity to review a

251 preliminary version of this manuscript for factual accuracy but the authors are solely

252 responsible for final content and interpretation. The authors received no financial support or

253 other form of compensation related to the development of the manuscript. The findings and

254 conclusions in this report are those of the authors.

255

256 **Competing interests**

257 E.R.A. and R.L.B. worked with Mologic (UK) to independently validate the SARS-CoV-2

258 ELISA at the Liverpool School of Tropical Medicine (LSTM). All other authors report no

259 potential conflicts.

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310     **Figure Legends**

311     **Figure 1. SARS-CoV-2 serological results from asymptomatic health care workers.** We

312     used a commercial ELISA to measure SARS-CoV-2 antibodies against Spike (S2) and

313     Nucleoprotein (N). OD, optical density.

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315     **Figure 2. Map of Blantyre showing geospatial distribution of seropositive results.** We

316     collected geocoordinate data for the place of residence of all study participants at recruitment.

317     The geocoordinates were combined with the ELISA assay results and plotted on the map of

318     Blantyre using R. Black dot, seronegative; Orange dot, indeterminate; Red dot, seropositive.

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335 **Tables**

336 **Table 1.** Study participant demographics

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| Characteristics | Number of participants | Proportion |
|---|---|---|
| **Gender** | | |
| Male | 236 | 47% |
| Female | 264 | 53% |
| **Age** | | |
| 20-29 | 209 | 42% |
| 30-39 | 170 | 34% |
| 40-49 | 86 | 17% |
| 50-59 | 28 | 6% |
| 60+ | 7 | 1 |
| **Occupation** | | |
| Clinical HCW | 331 | 66% |
| Non-Clinical HCW | 169 | 34% |
| **COVID-19 work** | | |
| Clinical | 205 | 41% |
| Non-Clinical | 43 | 9% |
| None | 252 | 50% |
| **Transport** | | |
| Public + Walking | 363 | 73% |
| Private | 137 | 27% |

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339 **COVID-19, Coronavirus Diseases of 2019; HCW, Health Care Workers**

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348 **Table 2.** Crude estimates of predicted mortality at the observed seroprevalence

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| Age | Population* (Blantyre) | Population* (Malawi) | Infection fatality rate † | Number of Infections | | Deaths | |
|---|---|---|---|---|---|---|---|
| | | | | Blantyre | Malawi | Blantyre | Malawi |
| **0-9 yrs** | 207,002 | 5,394,769 | 0.00% | 24,840 | 663,557 | 0 | 11 |
| **10-19 yrs** | 199,915 | 4,753,846 | 0.01% | 23,990 | 584,723 | 2 | 41 |
| **20-29 yrs** | 176,360 | 2,997,379 | 0.03% | 21,163 | 368,678 | 7 | 114 |
| **30-39 yrs** | 130,362 | 2,160,103 | 0.08% | 15,643 | 265,693 | 13 | 224 |
| **40-49 yrs** | 67,618 | 1,316,593 | 0.16% | 8,114 | 161,941 | 13 | 261 |
| **50-59 yrs** | 28,397 | 722,800 | 0.60% | 3,408 | 88,904 | 20 | 529 |
| **60-69 yrs** | 15,225 | 494,678 | 1.93% | 1,827 | 60,845 | 35 | 1,174 |
| **70-79 yrs** | 5,715 | 280,394 | 4.28% | 686 | 34,488 | 29 | 1,476 |
| **80+ yrs** | 2,001 | 152,762 | 7.80% | 240 | 18,790 | 19 | 1,466 |
| **Total** | **832,595** | **18,273,324** | | **99,911** | **2,247,619** | **138** | **5,295** |

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351 **\*2018 Population and Housing Census. † estimates derived from Verity, R. et al. 2020.**
352 **The total number of reported COVID-19 deaths on 16th July in Blantyre was 17 and in**
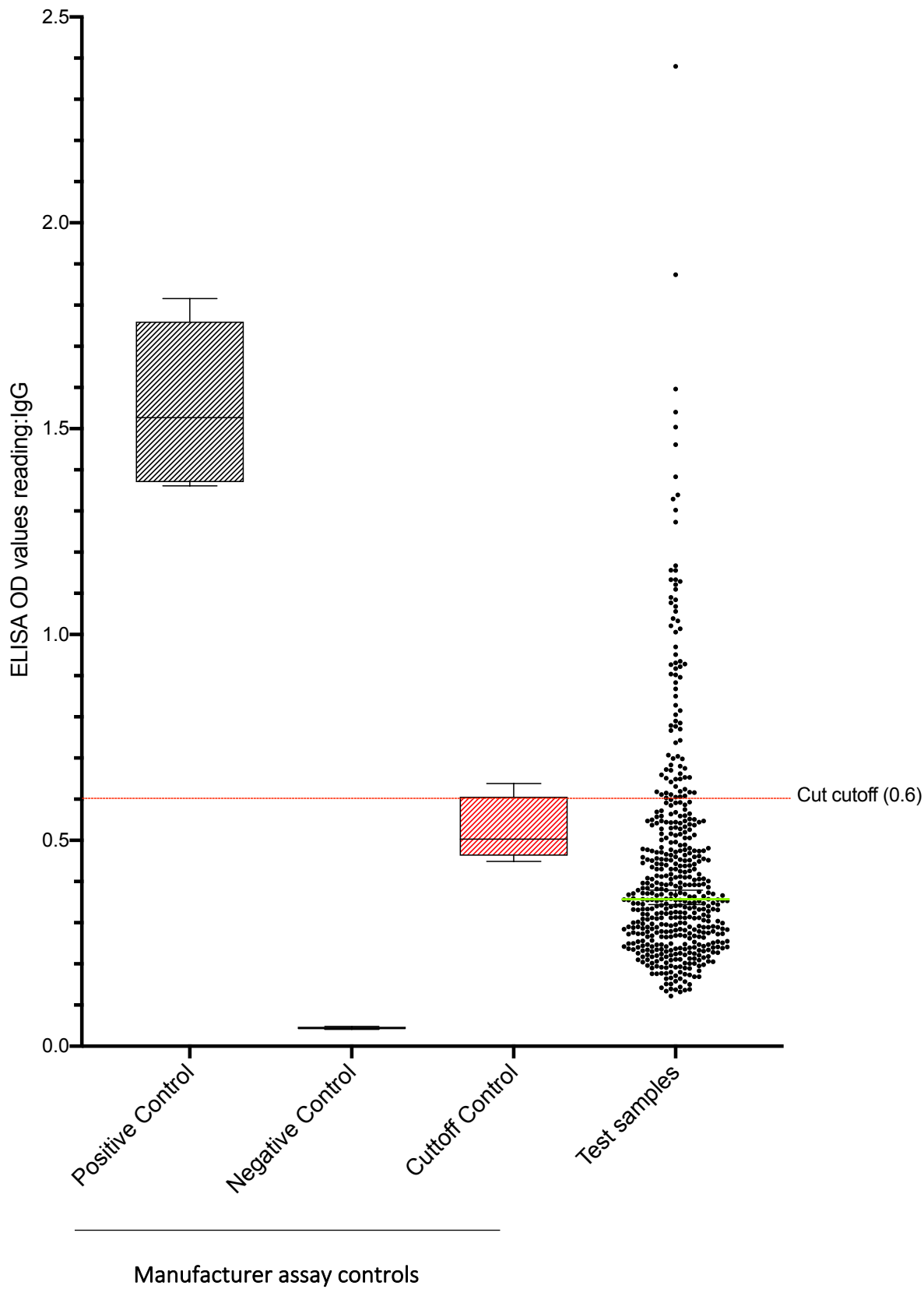353 **Malawi was 51.**

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**Figure 1.** SARS CoV-2 serological results from asymptomatic health care workers
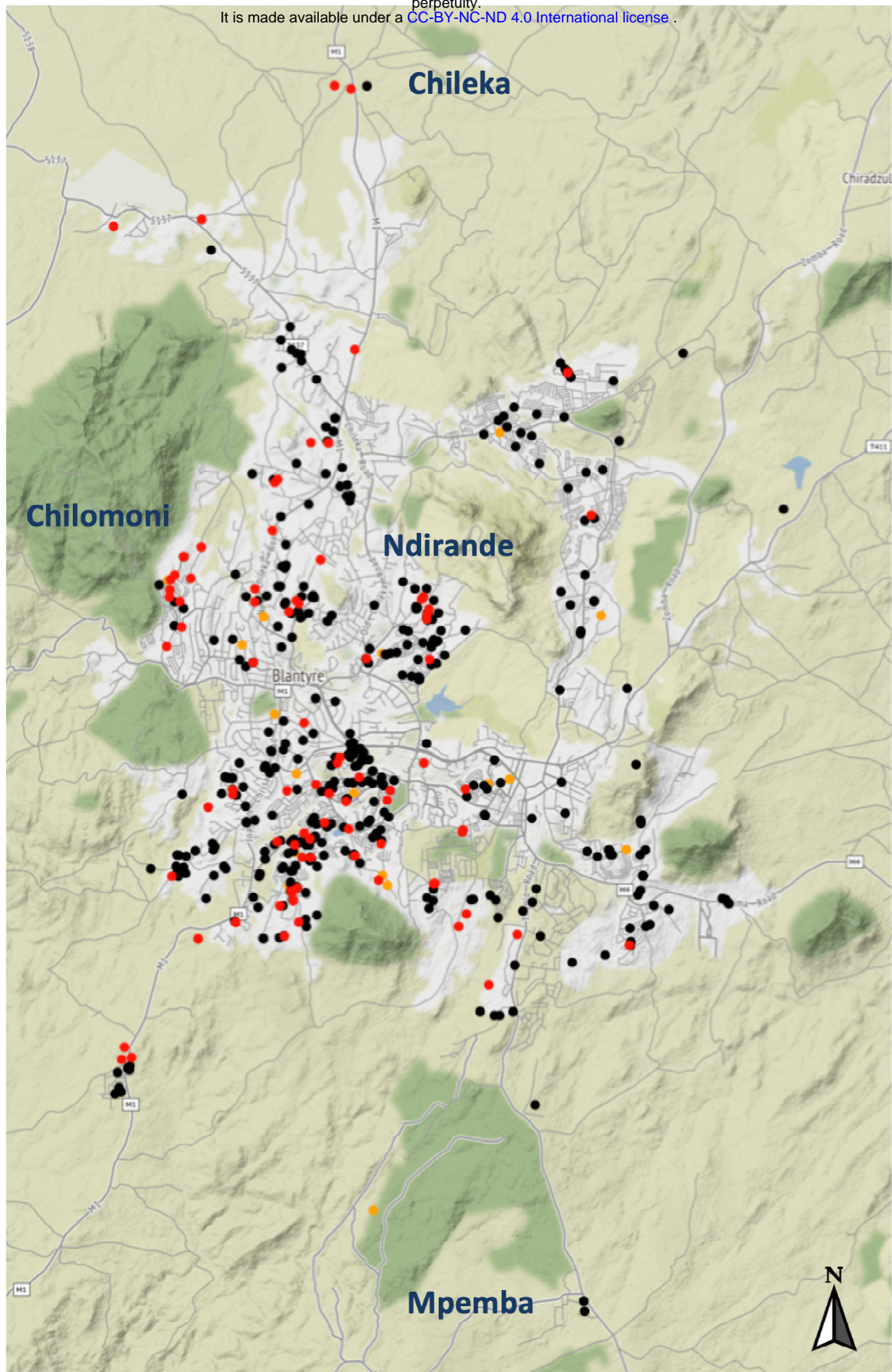
**Figure 2.** Map of Blantyre showing geospatial distribution of seropositive results